What is claimed is:

1. A method of purifying human acid a-glucosidase comprising: (a) applying a sample containing human acid a-glucosidase and contaminating proteins to an anion exchange or affinity column under conditions in which the a-glucosidase binds to the column; (b) collecting an eluate enriched in a-glucosidase from the anion exchange or affinity column; (c) applying the eluate to (i) a hydrophobic interaction column under conditions in which a-glucosidase binds to the column and then collecting a further eluate further enriched in a-glucosidase, or (ii) contacting the eluate with hydroxylapatite under conditions in which a-glucosidase does not bind to hydroxylapatite and then collecting the unbound fraction enriched in a-glucosidase.

2. The method of claim 2, wherein the column in steps (a) and (b) is an anion exchange column.

3. The method of claim 2 or claim 3, wherein the anion exchange column is Q-Sepharose.

4. The method of claim 4, wherein the sample is applied to the Q Sepharose column in low salt buffer and is eluted from the column in an elution buffer of higher salt concentration.

5. The method of claim 2 or claim 3, wherein the anion exchange column is copper chelating Sepharose.

6. The method of Claim 2, wherein the affinity column is lentil Sepharose.

7. The method of claim 2 or claim 3, wherein the hydrophobic interaction column is phenyl Sepharose.

8. The method of claim 2 or claim 3, wherein the hydrophobic interaction column is Source

~~Phenyl-15.~~

9. The method of claim 8, wherein the eluate is applied to the hydrophobic interaction column in a loading buffer of about 0.5 M ammonium sulphate and is eluted from the column with a low salt elution buffer.

10. The method of any one of claims 2 to 9, further comprising repeating steps (a) and (b) and/or (c) until the a-glucosidase has been purified to 95%, preferably 99%, more preferably 99.9% w/w pure.

11. The method of any one of claims 2 to 10, wherein the sample is milk produced by a transgenic mammal expressing the a-glucosidase in its milk.

12. The method of claim 11, wherein the transgenic mammal is a cow.

13. The method of claim 11, wherein the transgenic mammal is a rabbit.

14. The method of any one of claims 11 to 13, further comprising centrifuging the milk and removing fat leaving skimmed milk.

15. The method of claim 14, further comprising washing removed fat with aqueous solution, recentrifuging, removing fat and pooling supernatant with the skimmed milk.

16. The method of 15, further comprising removing caseins from the skimmed milk.

17. The method of claim 16, wherein the removing of caseins comprises a step selected from the group consisting of: high speed centrifugation followed by filtration; filtration using successively decreasing filter sizes; and cross-flow filtration.

~~18. The method of any preceding claim, wherein the sample has a volume of at least 100 liters.~~

19. At least 95%, preferably at least 99%, more preferably at least 99.9% w/w pure human acid a-glucosidase.

20. Human acid a-glucosidase substantially free of other biological materials.

21. Human acid a-glucosidase substantially free of contaminants.

sub a10

22. Human acid a-glucosidase of any one of claims 19-21 produced by the process of any one of claims 1-18.

23. A pharmaceutical composition for single dosage intravenous administration comprising at least 5mg/kg of at least 95%, preferably at least 99%, more preferably at least 99.9% (w/w) pure human acid aglucosidase.

sub a11

24. A pharmaceutical composition comprising human acid a- glucosidase as claimed in any one of claims 19-21.

25. Human acid a-glucosidase of any one of claims 19-21 for use as a pharmaceutical.

Subt C1

26. A method of treating a patient deficient in endogenous a- glucosidase, comprising administering a dosage of at least 5mg/kg of at least 95%, preferably at least 99%, more preferably at least 99.9% (w/w) pure human acid a-glucosidase intravenously to the patient, whereby the a-glucosidase is taken up by liver, heart and/or muscle cells of the patient.

sub a12

27. The use of human acid a-glucosidase of any one of claims 19-21 for the manufacture of a medicament for treatment of human acid a- glucosidase deficiency.

28. The use of human acid a-glucosidase of any one of claims 19-21 for the manufacture of a medicament for intravenous administration for the treatment of human acid a-glucosidase deficiency.

29. A method of purifying an heterologous protein from the milk of a transgenic animal comprising : a) contacting the transgenic milk or a transgenic milk fraction with a hydroxylapatite under conditions such that at least a substantial number of the milk protein

species other than the heterologous protein bind to the hydroxylapatite and the heterologous protein remains substantially unbound, and; b) removing the substantially unbound heterologous protein.

30. A method as claimed in claim 29, wherein the removal of the substantially unbound heterologous protein involves liquid flow through at least a portion of the hydroxylapatite.

31. A method as claimed in claim 30, wherein the liquid flow arises due to one or more forces selected from pumping, suction, gravity and centrifugal force.

sub a13

32. A method as claimed in any of claims 29 to 31 being a batch procedure.

33. A method as claimed in any of claims 29 to 31, wherein the hydroxylapatite is in the form of a column, optionally the method is a liquid column chromatography procedure.

34. A method as claimed in any of claims 29 to 33, wherein the heterologous protein ie selected from lactoferrin, transferrin, lactalbumin, factor IX, growth hormone, a-anti-trypsin, lactoferrin, transferrin, lactalbumin, coagulation factors such as factor VIII and factor IX, growth hormone, a-anti- trypsin, plasma proteins such as serum albumin, C1-esterase inhibitor and fibrinogen, collagen, immunoglobulins, tissue plasminogen activator, interferons, interleukins, peptide hormones, and lysosomal proteins such as a-glucosidase, a-L-iduronidase, iduronate-sulfate sulfatase, hexosaminidase A and B, ganglioside activator protein, arylsulfatase A and B, iduronate sulfatase, heparan N-sulfatase, galactoceramidase, a-galactosylceramidase A, sphingomyelinase, a-fucosidase, a-mannosidase, aspartylglycosamine amide hydrolase, acid lipase, N-acetyl-a-D-glycosamine-6-sulphate sulfatase, a-and ss-galactosidase, ss-glucuronidase, ss-mannosidase, ceramidase, galactocerebrosidase, a-N-acetylgalactosaminidase, and protective protein and others including allelic, cognate or induced variants as well as polypeptide fragments of the same.

35. A method as claimed in any of claims 29 to 24, ~~wherein the heterologous protein is not one~~ normally found in the milk of an animal.

36. A method of purifying human acid a-glucosidase comprising contacting a sample containing human acid a-glucosidase and contaminating proteins with hydroxylapatite under conditions in which aglucosidase does not bind to the hydroxylapatite and then collecting the unbound fraction enriched in a-glucosidase.

sub a'14

37. The method of claim 26, ~~wherein the hydroxylapatite is in the form of a column and the~~ unbound fraction is collected in the flow-through.

38. A method of purifying human acid a-glucosidase substantially as hereinbefore ~~described and~~ with reference to the examples and ~~accompanying~~ drawings.

39. Human acid a-glucosidase substantially as hereinbefore described and with reference to the examples and accompanying drawings.

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